



Is Cutibacterium (previously Propionibacterium) acnes a potential pathogenic factor in the aetiology of the skin disease progressive macular hypomelanosis?

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Title:

Is *Cutibacterium* (previously *Propionibacterium*) *acnes* a potential pathogenic factor in the aetiology of the skin disease progressive macular hypomelanosis?

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22 **Abstract**

23 Progressive macular hypomelanosis (PMH) is a skin condition that normally causes
24 symmetrically distributed hypopigmented macules on the front and back of the trunk, but
25 rarely the face. To date, the pathophysiology of the condition is not well understood, but a
26 role for the anaerobic skin bacterium *Cutibacterium* (previously *Propionibacterium*) *acnes* in
27 the development of the disease has been proposed due to its sole presence within lesional,
28 but not normal peri-lesional, skin. The success of antimicrobials in the treatment of PMH also
29 provides circumstantial evidence that this association may be causal, although this is still to
30 be proven. More recent culture and metagenomic typing studies indicate that strains of *C.*
31 *acnes* subsp. *elongatum* (type III) may be important in the aetiology of the condition, which
32 would help to explain why PMH does not normally affect the face since such strains are rarely
33 present there, and why no association between this condition and acne vulgaris is found;
34 acne appears to primarily involve type IA₁ strains from *C. acnes* subsp. *acnes* (type I). In this
35 review we summarise current knowledge on the relationship between *C. acnes* and PMH, and
36 re-examine previous challenges to the view that the bacterium plays a role in the condition
37 against the backdrop of newly emerged data.

38 1. Introduction

39 Progressive macular hypomelanosis (PMH) is a relatively uncommon dermatosis usually
40 characterised by ill-defined nummular, non-scaly and symmetric hypopigmented macules
41 that form predominantly on the front and back of the trunk, and without prior history of skin
42 injury, infection or inflammation (Fig. 1); these macules can be discrete or display confluence
43 when found in and around the midline¹. Like the very common skin condition acne vulgaris,
44 PMH normally affects adolescents and young adults where its cosmetic effects may have
45 social and psychological impacts^{1,2,3}. The disorder rarely affects the proximal extremities and
46 the skin of the face, which has been a key clinical feature^{1,3}. PMH is found worldwide and
47 affects different Fitzpatrick skin types as well as both sexes, but the disorder does appear to
48 be much more prevalent in females^{1,4-7}. PMH is often misdiagnosed as the fungal infection
49 pityriasis versicolor, pityriasis alba or post-inflammatory hypopigmentation, and can remain
50 stable, progress slowly over a long time period or, in some cases, spontaneously disappear
51 after mid-life^{1,3,4,8}. While the exact incidence of PMH within different populations is unclear, it
52 is likely to be an under-reported disorder as some cases may be misdiagnosed and not all
53 patients, particularly males, will see a clinician for treatment⁶. In this review, we evaluate the
54 strength of current evidence supporting a pathogenic role for the anaerobic bacterium
55 *Cutibacterium acnes* (previously *Propionibacterium acnes*) in the aetiology of PMH,
56 particularly in light of recent culture and metagenomic typing studies, and investigation of
57 porphyrin production by different strains of the bacterium.

58 2. Histological and electron microscopic features

59 Histologically, PMH does not appear to be associated with any significant abnormalities of
60 the dermis apart from a decrease in epidermal pigment, but a mild perifollicular infiltrate of
61 lymphocytes has been observed in some, but by no means all, lesional skin samples^{2,4,6-10}.

Detailed ultrastructural studies have provided evidence that PMH results from altered melanogenesis, leading to reduced pigmentation, and changes in melanosome size, aggregation, maturation and distribution^{2,3,6,8-9,11-12}. Furthermore, it does not appear that defects in melanosome degradation play a role in the pathophysiology of PMH as there is no evidence for disintegrated melanosomes in the lysosomal compartments of PMH lesions¹².

3. Evidence supporting a potential role for *Cutibacterium acnes* in the aetiology of PMH

While the underlying cause of PMH is still not clear, a pathogenic role for the Gram-positive, anaerobic bacterium *C. acnes* in the aetiology of the condition has been proposed, possibly via the production of a depigmenting factor or an agent that interferes with melanogenesis^{4,5}. *C. acnes* is part of the normal human microbiota and found predominately on the skin and mucosal surfaces. The organism is an opportunistic pathogen most noted for its association with acne vulgaris¹³, but has now also been linked to other human infections and conditions, including medical device and soft tissue infections^{14,15}, cervical disc disease¹⁶, prostate cancer¹⁷ and sarcoidosis¹⁸.

3.1 Culture and non-culture-based detection

Bacterial culture has demonstrated the sole presence and accumulation of *C. acnes* in biopsy samples of PMH lesions, but not biopsies of adjacent non-lesional skin from the same patient^{4,5} (Table 1). Furthermore, 16S rRNA-based quantitative real-time PCR detection of *C. acnes* has found a significantly greater incidence of the bacterium in lesional versus adjacent non-lesion skin of patients in respect to genome copy number, consistent with culture results and indicating enrichment in lesions⁵; in the latter case we can speculate that this may reflect localised perturbations in the skin environment that stimulate overgrowth. The presence of *C. acnes* within hypopigmented lesions from patients with PMH, but not normal pigmented skin from the trunk of the same patient, can also be observed upon Gram-staining, revealing

86 Gram-positive rods with a high population density^{1,4,9}. Furthermore, upon examination of the
87 skin in a dark room with UV radiation from a Wood's lamp, a punctiform orange-red follicular
88 fluorescence within hypopigmented spots is observed due to the presence of porphyrins
89 produced by the bacterium, such as coproporphyrin III; this fluorescence is absent in peri-
90 lesional normal skin^{1,4,7,10,12}. Interestingly, while this characteristic fluorescence of PMH lesions
91 upon Wood's lamp examination has been described in many studies, it has not been observed
92 in all (see section 5.1).

93 **3.2 Therapeutic success of antimicrobial-based treatments**

94 Treatments for PMH based on topical corticosteroids and topical or systemic antifungals
95 have not proved efficacious, but re-pigmentation of the skin can be achieved using ultraviolet
96 light A (UVA) or narrow-band UVB (NB-UVB)-based treatments, either as a monotherapy or in
97 combination with topical or oral antimicrobials^{1,6,9,10,19-24}. Such UV treatments are believed to
98 work by stimulating melanogenesis and, potentially, inhibition of *C. acnes* in the case of NB-
99 UVB (see section 5.3), but the results appear variable and in many cases are only temporary
100 leading to recurrence of the condition^{6,20,21,24}.

101 In a within-patient, left-right trunk comparison study, Relyeld et al.²⁵ demonstrated that
102 topical treatment with 5% benzoyl peroxide (BPO) (morning) and 1% clindamycin hydrogel
103 (night-time) in combination with UVA radiation (antibacterial therapy arm) was much
104 superior for re-pigmentation versus 0.05% fluticasone and UVA treatment only (anti-
105 inflammatory therapy arm). This appeared, therefore, to exclude the possibility that
106 treatment success was due solely to UVA treatment, and provided evidence to support a
107 bacterial role, such as *C. acnes*, in the pathophysiology of PMH. Furthermore, upon a 3-month
108 follow-up, PMH patients were still found to have retained their re-pigmentation, although
109 information on whether this persisted is not available. Since then, there have been many

110 studies investigating the effectiveness of treatments for PMH using topical antibiotic lotions
111 and BPO hydrogels (in combination or separately) alongside narrow-band UVB (NB-UVB)
112 treatment^{6,9,19,21,23}. Treatment success for PMH has also been achieved using the oral
113 tetracycline derivatives doxycycline, minocycline and lymecycline, used in the management of
114 acne, either with or without BPO²⁶⁻²⁹; this provides further circumstantial evidence that *C.*
115 *acnes* may contribute to the development of PMH. In particular, treatment of PMH with a
116 combination of oral lymecycline (300 mg/d) and topical 5% BPO was very successful leading
117 to repigmentation and maintenance during a 6-to-12 month follow-up period^{28,29}. Attempts
118 to treat PMH using oral isotretinoin have also been described in the literature, but the results
119 obtained have been variable³⁰⁻³¹.

120 **4. The hunt for a novel *Cutibacterium* species associated with PMH**

121 A conundrum in the proposal that *C. acnes* is the cause of PMH has been why the disorder,
122 unlike acne, rarely affects the face where levels of the bacterium are at their highest, and why
123 acne does not predispose individuals to PMH development. This led Relyveld et al.³² to
124 propose that the organism potentially causing PMH may not actually be *C. acnes*, but a closely
125 related *Cutibacterium* species indistinguishable by conventional phenotypic/ biochemical
126 methods.

127 **4.1 Amplified Fragment Length Polymorphism and 16SrRNA gene analysis**

128 Genetic analysis of skin biopsy-associated bacterial isolates collected from patients with
129 PMH and patients with acne by Amplified Fragment Length Polymorphism (AFLP) typing
130 identified three major genetic clusters that differed in their distribution between the two
131 conditions ($p < 0.01$; Freeman-Halton extension of Fisher's exact test) (Table 2)³². Of note was
132 the observation that isolates from DNA group 3, in contrast to the other DNA groups, were
133 only associated with PMH, but never acne (Fisher's exact test; $p < 0.01$), and analysis of

multiple bacterial colonies isolated from acne and PMH samples did not demonstrate the presence of mixed AFLP types. 16S rRNA gene sequencing revealed very high sequence identities between all clusters, with only a single nucleotide polymorphism (SNP) at position 827 separating DNA groups I and II³², which is a characteristic difference between the well described *C. acnes* type I (*C. acnes* subsp. *acnes*) and type II (*C. acnes* subsp. *defendens*) phylotypes³³, while group 3 isolates differed from group 1 due to a SNP at position 1243 (G1243A). While biochemical analysis with the rapid ID 32A multi-test identification system confirmed DNA groups 1 and 2 as *C. acnes* (99.9% certainty), isolates from DNA group 3 gave ambiguous results and could not be identified phenotypically despite the molecular results indicating a unique *C. acnes* cluster; 16S rRNA identity is not, however, always a guarantee of species identity, especially in the case of a recently diverged and very closely related sister taxon³⁴. This led to the proposal that organisms from DNA group 3 may represent a novel and very closely related bacterium from the genus *Cutibacterium*³².

4.2 PCR phylotyping and single- and multi-locus sequence type analysis

At the time of the original AFLP study of Relyveld et al.³², knowledge on the intraspecies diversity of *C. acnes* was only developing, as were the molecular methods for more detailed population genetic analysis of the bacterium. Today, our appreciation of *C. acnes* at the interspecies level is much more complete (Table 3), and specific molecular typing tools for the bacterium, particularly multiplex-PCR phylotyping, high-resolution single and multilocus sequence typing (HR-SLST and MLST, respectively) and ribotyping³⁵⁻³⁹, have also been established enabling researchers to deeper explore the association of specific lineages with skin health and disease.

Against this new landscape of understanding, and utilising the improved typing methods now available, Barnard et al.⁴⁰ conducted a population genetic analysis of *C. acnes* isolates

158 recovered from the lesional skin of patients with PMH. They demonstrated a strong statistical
159 association between strains from the more recently described type III phylogenetic lineage
160 (now known as *C. acnes* subsp. *elongatum*^{41,42}) and lesions, but not those representing other
161 phylogenetic groups, including those associated with acne (type IA₁). Strikingly, *in silico* 16S
162 rDNA SNP analysis revealed that the isolates from AFLP DNA group 3 (G1243A) previously
163 described in association with PMH were also consistent with the type III lineage (Fig. 2).
164 Furthermore, analysis of the biochemical phenotype of three representative type III strains
165 from PMH lesions using the Rapid ID 32A multi-tests identification system failed to correctly
166 identify the isolates as *C. acnes*, consistent with the previous results obtained for AFLP DNA
167 group 3 strains³².

168 A subsequent study by Peterson et al.²⁹ based on HR-SLST metagenomic analysis of skin
169 surface swabs taken from 24 PMH back lesions and adjacent non-lesional skin regions of eight
170 female patients, confirmed the association of type III strains with PMH. Interestingly,
171 treatment of patients using a combination of lymecycline (300 mg/d) and 5% BPO led to a
172 reduced proportion of type III within patients' samples, and a parallel reduction or
173 disappearance of their PMH lesions. In patients whose type III population was almost totally
174 eliminated there was almost no lesions remaining and the type distribution after treatment
175 generally reverted to that of controls (Fig. 1). Investigation of eight healthy female volunteers
176 also found that type III strains were more common on the back, especially the lower back, but
177 normally not present on the forehead or buccal mucosa; one patient was, however, found to
178 have a significant proportion of type III strains on their forehead despite, presumably, no
179 PMH lesions at this site (presence or absence of facial PMH was not definitely stated for this
180 patient). Unlike previous studies, a relatively high proportion of type III isolates was also
181 found on non-lesional skin, but this may have reflected issues around clear differentiation

182 between lesional and non-lesional sites using skin swab sampling as opposed to skin biopsy.

183 **5. Challenges to the proposal that *C. acnes* is involved in the aetiology of PMH**

184 **5.1 PMH in the apparent absence of lesional *C. acnes***

185 Despite independent studies highlighting a strong association of *C. acnes* with PMH, a
186 number of cases where *C. acnes* appears absent in lesional skin, as judged by Wood's lamp
187 examination, histological staining and, in some cases, microbiological culture from skin swabs
188 or biopsies have been reported^{6,8,43-44}. It is important, however, to note that a negative
189 Wood's lamp result does not confirm that *C. acnes* is absent, only that levels are below the
190 density detection limit ($\sim 10^3$ organisms)⁴⁵. Furthermore, a recent investigation found that
191 type II and III strains produce very low levels of porphyrin compared with type I organisms,
192 and that cultures of type II and III strains on solid media do not fluoresce upon Wood's lamp
193 illumination⁴⁶. This indicates that lesions dominantly or solely colonised with type III strains
194 may not have detectable follicular fluorescence. It also highlights that PMH lesions normally
195 appear to have a mixed phylotype composition containing at least fluorescent type I strains,
196 as well as type III in the majority of instances. The absence of mixed types and the detection
197 of mostly type III strains based on previously described culture-based studies of lesional skin
198 biopsies may, therefore, reflect the differential growth of dominant clones of this subspecies
199 present in high numbers.

200 **5.2 Rare occurrence of PMH on the face where *C. acnes* type III are normally absent.**

201 A lack of facial involvement in PMH, despite high concentrations of *C. acnes* at this site,
202 has been one of the biggest challenges to the view the bacterium has a role in the
203 development of this condition. The observation of an association between *C. acnes* type III
204 strains and PMH does, however, help to explain, at least in part, this intriguing clinical feature
205 of the disease since type III strains normally appear to be absent or found in very low

abundance on the face of most individuals³⁸. While a recent study reporting four adult cases with apparent facial PMH, in addition to trunk, arm and leg lesions, appears inconsistent with this view, no microbiological analysis was described for these patients⁴⁷. As a result, conclusions regarding the potential role of the bacterium in these specific facial cases of PMH cannot be completely dismissed. It was interesting to note that the patients were much older (40-65 years) than normally seen and it is currently unclear how the distribution of *C. acnes* phylogroups and specific strain types on the skin may modify as we age; we can speculate that in some older individuals type III strains may become more abundant on the face due to age-related changes in cutaneous physiology that influence bacterial diversity. The previous observation of a PMH patient with significant levels of type III on their forehead does highlight that, while uncommon, the bacterium can indeed be present on the face of some individuals²⁹ (section 4.2). However, the presumed presence of only truncal lesions on this patient does complicate the view that a lack of facial PMH is solely down to the absence of type III organisms at this skin site. Other factors, including the nature of the strain type(s) present, their abundance and interaction with other microbiota, may well be important factors, alongside host response and other variables.

5.3 Antibacterial therapy versus phototherapy

A number of studies have challenged the original findings of Relyeld et al.²⁵ in regards to the effectiveness of antimicrobial treatment and phototherapy versus phototherapy alone. In particular, Sim et al.²¹ and Selim et al.⁶ did not find any significant difference in repigmentation of PMH lesions using daily topical 5% BPO and 1% clindamycin antimicrobial treatments with NB-UVB versus NB-UVB monotherapy. Furthermore, in many cases recurrence of the condition occurred, although some patients retained a degree of clinical improvement. While such observations question the pathogenic role for *C. acnes* in PMH, a

key difference between these studies and that of Relyeld et al.²⁵ relates to the use of NB-UVB rather than UVA plus psoralen (PUVA). NB-UVB has been shown, *in vitro*, to have antibacterial effects on cutibacteria which is not observed with UVA, potentially explaining the contradictory results^{48,49}. It is interesting to note, however, that in the study of Selim et al.⁶ only two PMH patients had hypopigmented lesions that demonstrated fluorescence under a Wood's lamp indicating absent or low levels of *C. acnes*, or colonization with low porphyrin-producing strains, while data from Sim et al.²¹ in relation to Wood's lamp analysis was not described. In contrast, Hassan et al.⁹ found that topical (2% erythromycin lotion) and systemic (100 mg doxycycline b.i.d) antimicrobial treatments alongside NB-UVB for 3 months did give superior results compared to NB-UVB alone, and with no relapse in a 6 month follow up period.

6. Future research.

Additional studies are clearly needed to further dissect the underlying biological mechanisms driving the development of PMH. To date, our understanding of the underlying biology of type III strains and their interaction with other microbiota, alongside their niche requirements and capacity to cause disease, remains unclear, but an inflammatory phenotype has been observed, as well as the presence and absence of unique genomic elements when compared to other phylotypes^{40,50}. It will be important to determine whether *C. acnes*, and particularly type III strains, have the capacity to interfere with melanogenesis via a depigmenting factor(s) or stimulation of a specific host response; initially, this could be achieved using appropriate *in vitro* cell culture models to study host-interacting properties. It is also interesting that some patients confuse PMH with leprosy, a chronic granulomatous disease caused by another intracellular bacterium, *Mycobacterium leprae*¹. In particular, the tuberculoid form of the disease is characterised by a very small number of scaly, well defined

254 hypopigmented macules of varying symmetry on the skin, although poorly defined macules
255 with mild hypopigmentation and erythema are also present in lepromatous forms⁵¹. Previous
256 work has suggested this reflects marked reductions in the number of normal melanocytes in
257 the lesions and the presence of atrophic melanocytes with reduced activity, while other
258 studies suggest it may reflect defective transfer of melanosomes from melanocytes to
259 keratinocytes^{52,53}. In the tuberculoid form of the condition, acid-fast bacilli are rarely found,
260 which may be a relevant observation when considering PMH lesions in the apparent absence
261 of *C. acnes* (see section 5.1). While hypomelanosis disorders can also be caused by other
262 types of microorganisms, such as fungi and yeasts, hypopigmentation in leprosy appears
263 more noteworthy in the context of PMH given that mycobacteria and cutibacteria are
264 distantly related actinomycetes, and have also been linked to another granulomatous
265 disease, sarcoidosis⁵⁴. While highly speculative, it may be that the hypopigmentation
266 observed in both conditions is driven by some shared or similar characteristic of these
267 bacteria (secretory or host-response). Previous studies on leprosy pathogenesis may,
268 therefore, have informative aspects for researchers interested in future PMH studies, despite
269 the many obvious differences between the two diseases.

270 The observation of PMH in twins, along with our current understanding of the
271 epidemiology of the condition, also hints at a multifactorial inheritance aetiology driven by
272 both genetics and environmental factors that may include specific strains of *C. acnes* and
273 hormonal influences given its apparent increased rate in females and description of an
274 acceleration of hypopigmentation in one patient after pregnancy⁸. The penetrance of PMH is,
275 therefore, likely to be an interplay of these different influences, and further studies of genetic
276 factors that may influence development of the disease and its clinical course should also be
277 an important area of focus. We also need to better understand the pathophysiology of those

278 rare cases where PMH involves the face, as well as any differences that occur in the
279 development of the condition in the presence and apparent absence of lesional *C. acnes*.

280 **7. Conclusion**

281 While a number of independent studies have found a strong association between *C. acnes*
282 type III and PMH, a definite causal role is still to be determined. Nevertheless, the
283 demonstration that type III strains are associated with the condition does help to explain, at
284 least partly, the observations that PMH does not normally affect the face, nor is linked to the
285 development of acne. Although reports of PMH in the apparent absence of *C. acnes* do
286 complicate our understanding of the bacterium's role in the disease, further studies are
287 required to definitely confirm this. It may be that *C. acnes* is only one of a number of different
288 factors that can influence the development of PMH or that, in some instances, the bacterium
289 initiates a biological response that leads to hypopigmentation, even after it becomes no
290 longer detectable within lesions.

291 **REFERENCES:**

- 292 1. Relyveld GN, Menke HE, Westerhof W. Progressive macular hypomelanosis. An overview.
293 *Am J Clin Dermatol* 2007; **8**: 13-19.
- 294 2. Kumarasinghe SPW, Tan SH, Thng S, et al. Progressive macular hypomelanosis in
295 Singapore: a clinico-pathological study. *Int J Dermatol* 2006; **45**: 737-743.
- 296 3. Guillet G, Helenon R, Gauthier Y, Surleve-Bazeil le JE, Plantin P, Sassolas B. Progressive
297 macular hypomelanosis of the trunk: primary acquired hypopigmentation. *J Cutan Pathol*
298 1998; **15**: 286-289.
- 299 4. Westerhof W, Relyveld GN, Kingswijk, MM. *Propionibacterium acnes* and pathogenesis of
300 macular hypomelanosis. *Arch Dermatol* 2004; **140**: 210-214.
- 301 5. Cavalcanti SM, de França ER, Lins AK, Magalhães M, de Alencar ERB, Magalhães V.
302 Investigation of *Propionibacterium acnes* in progressive macular hypomelanosis using real-
303 time PCR and culture. *Int J Dermatol* 2011; **50**: 1347-1352.
- 304 6. Selim MK, Ahmed El-SF, Abdelgawad MM, El-Kamel MF. Progressive macular
305 hypomelanosis among Egyptian patients: a clinicopathological study. *Dermatol Pract*
306 *Concept* 2011; **1**: 5-11.
- 307 7. Martinez-Martinez ML, Azaña-Defez JM, Rodríguez-Vázquez M, Faura-Berruga C, Escario-
308 Travesedo E. Progressive macular hypomelanosis. *Pediatr Dermatol* 2012; **29**: 460-462.
- 309 8. Neynaber S, Kirschner C, Kamann S, Plewig G, Flaig MJ. Progressive macular
310 hypomelanosis: a rarely diagnosed hypopigmentation in caucasians. *Dermatol Res Pract*
311 2009; **2009**: 607682.
- 312 9. Hassan AM, El-Badawi MA, Abd-Rabbou FA, Gamei MM, Moustafa KA, Almokadem AH.
313 Progressive macular hypomelanosis pathogenesis and treatment: a randomized clinical
314 trial. *J Microsc Ultrastruc* 2014; **2**: 205-216.

- 315 10. Hwang SW, Hong SK, Kim SH, et al. Progressive macular hypomelanosis in Korean
316 patients: a clinicopathologic study. *Ann Dermatol* 2009; **21**: 261-267.
- 317 11. Relyveld GN, Dingemans KP, Menke HE, Bos JD, Westerhof W. Ultrastructural findings in
318 progressive macular hypomelanosis indicate decreased melanin production. *J Eur Acad*
319 *Dermatol Venereol* 2008; **22**: 568-754.
- 320 12. Wu XG, Xu AE, Song XZ, Zheng JH, Wang P, Shen H. Clinical, pathologic, and
321 ultrastructural studies of progressive macular hypomelanosis. *Int J Dermatol* 2010; **49**:
322 1127-1132.
- 323 13. McLaughlin J, Watterson S, Layton AM, Bjourson AJ, Barnard E, McDowell A.
324 *Propionibacterium acnes* and acne vulgaris: new insights from the integration of
325 population genetic, multi-omic, biochemical and host-microbe studies. *Microorganisms*
326 2019; **7**: 128.
- 327 14. Piper KE, Jacobson MJ, Cofield RH, et al. Microbiologic diagnosis of prosthetic shoulder
328 infection by use of implant sonication. *J Clin Microbiol* 2009; **47**: 1878–1884.
- 329 15. Gao TW, Li CY, Zhao XD, Liu YF. Fatal bacteria granuloma after trauma: a new entity. *Br J*
330 *Dermatol* 2002; **147**: 985-993.
- 331 16. Stirling A, Worthington T, Rafiq M, Lambert PA, Elliott TS. Association between sciatica
332 and *Propionibacterium acnes*. *Lancet* 2001; **357**: 2024-2025.
- 333 17. Cohen RJ, Shannon BA, McNeal JE, Shannon T, Garrett KL. *Propionibacterium*
334 *acnes* associated with inflammation in radical prostatectomy specimens: A possible link
335 to cancer evolution? *J Urol* 2005; **173**: 1969–1974.
- 336 18. Eishi Y. Etiologic link between sarcoidosis and *Propionibacterium acnes*. *Respir Investig*
337 2013; **51**: 56–68.

- 338 19. Wu XG, Xu AE, Luo XY, Song XZ. A case of progressive macular hypomelanosis successfully
339 treated with benzoyl peroxide plus narrow-band UVB. *J Dermatolog Treat* 2010; **21**: 367-
340 368.
- 341 20. Menke HE, Ossekoppele R, Dekker SK, et al. Nummulaire en confluierende hypomelanosis
342 van de romp. *Ned Tijdsch Dermatol Venereol* 1997; **7**: 117-122.
- 343 21. Sim JH, Lee DJ, Lee JS, Kim YC. Comparison of the clinical efficacy of NBUVB and NBUVB
344 with benzoyl peroxide/ clindamycin in progressive macular hypomelanosis. *J Eur Acad*
345 *Dermatol Venereol* 2011; **25**: 1318-1323.
- 346 22. Chung YL, Goo B, Chung WS, Lee GS, Hann SK. A case of progressive macular
347 hypomelanosis treated with narrow-band UVB. *J Eur Acad Dermatol Venereol* 2007; **21**:
348 1007-1009.
- 349 23. Erpolat S, Gorpelioglu C, Sarifakioglu E. A case of progressive macular hypomelanosis
350 treated with 1% topical clindamycin lotion and narrow-band ultraviolet B. *Photodermatol*
351 *Photoimmunol Photomed* 2010; **26**, 277-278.
- 352 24. Kim MB, Kim GW, Cho HH, et al. Narrowband UVB treatment of progressive macular
353 hypomelanosis. *J Am Acad Dermatol* 2011; **66**, 598-605.
- 354 25. Relyveld GN, Kingswijk MM, Reitsma JB, Menke HE, Bos JD, Westerhof W. Benzoyl
355 peroxide/clindamycin/ ultraviolet A is more effective than fluticasone/ultraviolet A in
356 progressive macular hypomelanosis: a randomised study. *J Am Acad Dermatol* 2006; **55**,
357 836-843.
- 358 26. Perman M, Sheth P, Lucky AW. Progressive macular hypomelanosis in a 16-year old.
359 *Pediatr Dermatol* 2008; **25**, 63-65.

- 360 27. de Almeida ART, Bedani TP, Debs EAF, Ferreira JAD. Estudo piloto para avaliar a eficácia
361 da minociclina no tratamento da hipomelanose macular progressiva (HMP) *Surg Cosmetic*
362 *Dermatol* 2009; **1**, 25–28.
- 363 28. Cavalcanti SMM, Querino MCD, Magalhaes V, Franca ER, Magalhães M, Alencar E. The
364 use of lymecycline and benzoyl peroxide for the treatment of progressive macular
365 hypomelanosis: a prospective study. *An Bras Dermatol* 2011; **86**, 813–814.
- 366 29. Peterson RLW, Scholz CFP, Jenson A, Brüggemann H, Lomholt HB. *Propionibacterium*
367 *acnes* phylogenetic type III is associated with progressive macular hypomelanosis. *Eur J*
368 *Microbiol Immunol (Bp)* 2017; **7**, 37-45.
- 369 30. Kim YJ, Lee DY, Lee JY, Yoon TY. Progressive macular hypomelanosis showing excellent
370 response to oral isotretinoin. *J Dermatol* 2012; **39**, 937-938.
- 371 31. Damevska K, Pollozhani N, Neloska L, Duma S. Unsuccessful treatment of progressive
372 macular hypomelanosis with oral isotretinoin. *Dermatol Ther* 2017; **30**, e12514.
- 373 32. Relyveld GN, Westerhof W, Woudenberg J, et al. Progressive macular hypomelanosis is
374 associated with a putative *Propionibacterium* species. *J Invest Dermatol* 2010; **130**, 1182-
375 1184.
- 376 33. McDowell A, Valanne S, Ramage G, et al. *Propionibacterium acnes* types I and II represent
377 phylogenetically distinct groups. *J Clin Microbiol* 2005; **43**, 326-334.
- 378 34. Fox GE, Wisotzkey JD, Jurtshuk Jr P. How close is close: 16s rRNA sequence identity may
379 not be sufficient to guarantee species identity. *Int J Syst Bacteriol* 1992; **42**, 166-170.
- 380 35. Barnard E, Nagy I, Hunyadkürti J, Patrick S, McDowell A. Multiplex touchdown PCR for
381 rapid typing of the opportunistic pathogen *Propionibacterium acnes*. *J Clin Microbiol*
382 2015; **53**, 1149-1155.

36. Scholz CFP, Jensen A, Lomholt HB, Brüggemann H, Kilian M. A novel high-resolution single locus sequence typing scheme for mixed populations of *Propionibacterium acnes* *in vivo*. *PLoS ONE* 2014; **9**, e104199

37. McDowell A, Nagy I, Magyari M, Barnard E, Patrick S. The opportunistic pathogen *Propionibacterium acnes*: insights into typing, human disease, clonal diversification and camp factor evolution. *PLoS ONE* 2013; **8**, e70897.

38. Fitz-Gibbon S, Tomida S, Chiu BH, et al. *Propionibacterium acnes* strain populations in the human skin microbiome associated with acne. *J Invest Dermatol* 2013; **133**, 2152-2160.

39. McDowell A. Over a Decade of *recA* and *tly* gene sequence typing of the skin bacterium *Propionibacterium acnes*: what have we learnt? *Microorganisms* 2017; **6**, 1.

40. Barnard E, Liu J, Yankova E, et al. Strains of the *Propionibacterium acnes* type III lineage are associated with the skin condition progressive macular hypomelanosis. *Sci Rep* 2016; **6**, 31968.

41. McDowell A, Perry AL, Lambert PA, Patrick S. A new phylogenetic group of *Propionibacterium acnes*. *J Med Microbiol* 2008; **57**, 218-224.

42. McDowell A, Barnard E, Liu J, Li H, Patrick S. Emendation of *Propionibacterium acnes* subsp. *acnes* (Deiko et al. 2015) and proposal of *Propionibacterium acnes* type II as *Propionibacterium acnes* subsp. *defendens* subsp. nov. *Int J Syst Evol Microbiol* 2016; **66**, 5358-5365.

43. Kuznetsov AV, Flaig MJ, Ruzicka T, Herzing T. Progressive macular hypomelanosis Guillet-Hélénon: structural and immunohistochemical findings. *J Clin Pathol* 2011; **64**, 734-736.

44. Wen LL, Wang T, Yang C, Yang S, Cui Y, Zhang XJ. Progressive macular hypomelanosis with asymmetrically distributed lesions. *Chin Med J (Engl)* 2013; **126**, 3591.

- 407 45. McGinley KJ, Webster GF, Leyden JJ. Facial follicular porphyrin fluorescence: correlation
408 with age and density of *Propionibacterium acnes*. *Br J Dermatol* 1980; **102**, 437-441.
- 409 46. Barnard E, Johnson T, Ngo T, et al. Porphyrin production and regulation in cutaneous
410 propionibacteria. *mSphere* 2020; **5**, e00793-19.
- 411 47. Wang K, Nassef Y, Sahu J, Hermes H, Schwartz LR. Facial involvement in progressive
412 macular hypomelanosis. *Cutis* 2018; **101**, 297-300.
- 413 48. Fluhr JW, Gloor M. The antimicrobial effect of narrow-band UVB (313 nm) and UVA1
414 (345-440 nm) radiation *in vitro*. *Photodermatol Photoimmunol Photomed* **1997**; **13**, 197-
415 201.
- 416 49. Kalayciyan A, Oguz O, Bahar H, Torun MM, Aydemir EH. *In vitro* bactericidal effect of low-
417 dose ultraviolet B in patients with acne. *J Eur Acad Dermatol Venereol* **2002**; **16**, 642-643.
- 418 50. Jasson F, Nagy I, Knol AC, Zuliani T, Khammari A, Dréno B. Different strains of
419 *Propionibacterium acnes* modulate differently the cutaneous innate immunity. *Exp*
420 *Dermatol* 2013; **9**, 587-592.
- 421 51. Walker SL, Lockwood DNJ. The clinical and immunological features of leprosy. *Br Med Bull*
422 2006; **77-78**, 103–121.
- 423 52. Job CK, Nayar A, Narayanan JS. Electronmicroscopic study of hypopigmented lesions in
424 leprosy. A preliminary report. *Br J Dermatol* 1972; **87**, 200-212.
- 425 53. Westerhof W. A possible dysfunction of melanosome transfer in leprosy: an electron-
426 microscopic study. *Acta Derm Venereol* 1977; **57**, 297-304.
- 427 54. Oswald-Richter KA, Beachboard DC, Seeley EH. *et al.* Dual analysis for mycobacteria and
428 propionibacteria in sarcoidosis BAL. *J Clin Immunol* 2012; **32**, 1129–1140.

Table 1. Key studies demonstrating an association between *C. acnes* and PMH based on culture analysis of lesional and adjacent non-lesional skin biopsies.

Study	M:F ^a	Biopsy	Lesional skin		Non-lesional skin		p-value ^b
			+	-	+	-	
Westerhof et al.	0:8	2 mm	7	1	1	7	0.04
Cavalcanti et al.	9:27	4 mm	33	2	4	31	<0.001
Total	9:35	-	40	3	5	38	<0.0001

^aMale:Female ratio

^bStatistical analysis was performed using McNemars test.

Table 2. Association of *C. acnes* AFLP genetic groups with acne and PMH

Disorder	AFLP analysis ^a			Total
	DNA group 1	DNA group 2	DNA group 3	
Acne	9	2	0	11
PMH	6	0	8	14
Total^b	15	2	8	25

^aData taken from the study of Relyveld et al.³²

^bp<0.01 (Freeman-Halton extension of Fisher's exact test) for differences between acne and PMH in regards to DNA group distribution.

Table 3. Association of *C. acnes* phylotype and subspecies status with AFLP and other typing methods.

Phylotype	Subspecies	AFLP typing group ^a	<i>recA</i> typing phylotype	MLST ₈ CC ^b	Ribotypes ^c
IA ₁	<i>acnes</i>	1	IA ₁ /IB ^d	CC1; CC3; CC4	RT1; RT5; RT532
IA ₂	<i>acnes</i>	1	IB	CC2	RT3; RT16
IB	<i>acnes</i>	1	IB	CC5	RT1
IC	<i>acnes</i>	1	IC	CC107	RT5
II	<i>defendens</i>	2	II	CC6; CC30; CC71, CC72	RT2; RT6
III	<i>elongatum</i>	3	III	CC77	RT9

^aAFLP group from the study of Relyveld et al.³²

^bCC= clonal complex (<https://pubmlst.org/cacnes/>)

^cRibotypes based on the study of Fitz-Gibbon et al.³⁸

^dIA₁ = CC1 and CC3; IB = CC4.

FIGURE LEGENDS:

Figure 1. Clinical responses of PMH lesions to antimicrobial treatment. Lesional skin on the back of two patients before (a and c) and after (b and d) daily treatment with lymecycline (300 mg/d) and BPO washes for 3 months. Figure and modified legend are from Petersen et al.²⁹.

Figure 2. Alignment of the 16S rDNA sequence from strain ATCC6919 (type IA₁), KPA171202 (type IB) and NCTC10390 (type II) versus type III isolates. The 16S rDNA G>A SNP described by Relyveld et al.³² as a genetic marker of AFLP DNA group 3 strains is highlighted. This SNP was present in eight of the 10 type III isolates analysed, but absent in type strains from the other major *C. acnes* lineages. Figure and modified legend are from Barnard et al.⁴⁰